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(54) **Long-lasting aqueous dispersions or suspensions of pressure-resistant gas-filled microvesicles and methods for the preparation thereof**

Langhaltende wässrige Dispersionen oder Suspensionen von druckfesten, gasgefüllten Mikrovesikeln und Verfahren zu ihrer Herstellung

Dispersions ou suspensions aqueuses de longue durée des microvésicules résistant à la pression et remplis de gaz et procédés pour leur préparation

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(56) References cited:
EP-A- 0 324 938
EP-A- 0 327 490
EP-A- 0 357 163
EP-A- 0 441 468
EP-A- 0 458 745
WO-A-91/15244
WO-A-92/17212
WO-A-92/17213

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Description

Technical Field

The present invention concerns stable dispersions or compositions of gas filled microvesicles in aqueous carrier liquids. These dispersions are generally usable for most kinds of applications requiring gases homogeneously dispersed in liquids. One notable application for such dispersions is to be injected into living beings, for instance for ultrasonic echography and other medical applications. The invention also concerns the methods for making the foregoing compositions including some materials involved in the preparations, for instance pressure-resistant gas-filled microbubbles, microcapsules and microballoons.

Background of Invention

It is well known that microbodies or microglobules of air or gas (defined here as microvesicles), e.g. microbubbles or microballoons, suspended in a liquid are exceptionally efficient ultrasound reflectors for echography. In this disclosure the term of "microbubble" specifically designates hollow spheres or globules, filled with air or a gas, in suspension in a liquid which generally result from the introduction therein of air or gas in divided form, the liquid preferably also containing surfactants or tensides to control the surface properties and the stability of the bubbles. The term of "microcapsule" or "microballoon" designates preferably air or gas-filled bodies with a material boundary or envelope, i.e. a polymer membrane wall. Both microbubbles and microballoons are useful as ultrasonic contrast agents. For instance injecting into the bloodstream of living bodies suspensions of air-filled microbubbles or microballoons (in the range of 0.5 to 10 μm) in a carrier liquid will strongly reinforce ultrasonic echography imaging, thus aiding in the visualization of internal organs. Imaging of vessels and internal organs can strongly help in medical diagnosis, for instance for the detection of cardiovascular and other diseases.

The formation of suspensions of microbubbles in an injectable liquid carrier suitable for echography can be produced by the release of a gas dissolved under pressure in this liquid, or by a chemical reaction generating gaseous products, or by admixing with the liquid soluble or insoluble solids containing air or gas trapped or adsorbed therein.

For instance, in US-A-4,446,442 (Schering), there are disclosed a series of different techniques for producing suspensions of gas microbubbles in a sterilized injectable liquid carrier using (a) a solution of a tenside (surfactant) in a carrier liquid (aqueous) and (b) a solution of a viscosity enhancer as stabilizer. For generating the bubbles, the techniques disclosed there include forcing at high velocity a mixture of (a), (b) and air through a small aperture; or injecting (a) into (b) shortly before use together with a physiologically acceptable gas; or adding an acid to (a) and a carbonate to (b), both components being mixed together just before use and the acid reacting with the carbonate to generate CO_2 bubbles; or adding an over-pressurized gas to a mixture of (a) and (b) under storage, said gas being released into microbubbles at the time when the mixture is used for injection.

EP-A-131,540 (Schering) discloses the preparation of microbubble suspensions in which a stabilized injectable carrier liquid, e.g. a physiological aqueous solution of salt, or a solution of a sugar like maltose, dextrose, lactose or galactose, is mixed with solid microparticles (in the 0.1 to 1 μm range) of the same sugars containing entrapped air. In order to develop the suspension of bubbles in the liquid carrier, both liquid and solid components are agitated together under sterile conditions for a few seconds and, once made, the suspension must then be used immediately, i.e. it should be injected within 5-10 minutes for echographic measurements; indeed, because they are evanescent, the bubble concentration becomes too low for being practical after that period.

In an attempt to cure the evanescence problem, microballoons, i.e. microvesicles with a material wall, have been developed. As said before, while the microbubbles only have an immaterial or evanescent envelope, i.e. they are only surrounded by a wall of liquid whose surface tension is being modified by the presence of a surfactant, the microballoons or microcapsules have a tangible envelope made of substantive material, e.g. a polymeric membrane with definite mechanical strength. In other terms, they are microvesicles of material in which the air or gas is more or less tightly encapsulated.

For instance, US-A-4,276,885 (Tickner et al.) discloses using surface membrane microcapsules containing a gas for enhancing ultrasonic images, the membrane including a multiplicity of non-toxic and non-antigenic organic molecules. In a disclosed embodiment, these microbubbles have a gelatine membrane which resists coalescence and their preferred size is 5-10 μm. The membrane of these microbubbles is said to be sufficiently stable for making echographic measurements.

Air-filled microballoons without gelatin are disclosed in US-A-4,718,433 (Feinstein). These microvesicles are made by sonication (5 to 30 kHz) of protein solutions like 5% serum albumin and have diameters in the 2-20 μm range, mainly 2-4 μm. The microvesicles are stabilized by denaturation of the membrane forming protein after sonication, for instance by using heat or by chemical means, e.g. by reaction with formaldehyde or glutaraldehyde. The concentration of stable microvesicles obtained by this technique is said to be about 8×10^6/ml in the 2-4 μm range, about 10^6 /ml in the 4-5 μm range and less than 5×10^5 in the 5-6 μm range. The stability time of these microvesicles is said to be 48 hrs or longer

and they permit convenient left heart imaging after intravenous injection. For instance, the sonicated albumin microbubbles when injected into a peripheral vein are capable of transpulmonary passage. This results in echocardiographic opacification of the left ventricle cavity as well as myocardial tissues.

Recently, still further improved microballoons for injection ultrasonic echography have been reported in EP-A-324.938 (Widder). In this document there are disclosed high concentrations (more than 10^8/ml) of air-filled protein-bounded microvesicles of less than 10 μm which have life-times of several months or more. Aqueous suspensions of these microballoons are produced by ultrasonic cavitation of solutions of heat denaturable proteins, e.g. human serum albumin, which operation also leads to a degree of foaming of the membrane-forming protein and its subsequent hardening by heat. Other proteins such as hemoglobin and collagen were also said to be convenient in this process. The high storage stability of the suspensions of microballoons disclosed in EP-A-0 324 938 enables them to be marketed as such, i.e. with the liquid carrier phase, which is a strong commercial asset since preparation before use is no longer necessary.

Similar advantages have been recently discovered in connection with the preparation of aqueous microbubble suspensions, i.e. there has been discovered storage-stable dry pulverulent composition which will generate long-lasting bubble suspensions upon the addition of water. This is being disclosed in Application WO-A-91/15244 where liposomes comprising membrane-forming lipids are freeze-dried, and the freeze-dried lipids, after exposure to air or a gas for a period of time, will produce long-lasting bubble suspensions upon simple addition thereto of an aqueous liquid carrier. Actually, this reference discloses a contrast agent for ultrasonic imaging of human or animal body comprising a suspension of air or gas microbubbles in which the microbubbles are stabilised by at least one film forming surfactant at least partially in lamellar or laminar form. The gases in the microbubbles include innocuous physiologically acceptable gases like CO_2, nitrogen, N_2O, methane, butane, freon and mixtures thereof, and radioactive gases such as xenon or krypton which are of interest in nuclear medicine for blood circulation measurements, lung scintillography etc.

EP-A-0 458 745 (Sintetica) discloses air or gas filled microballoons bounded by an interfacially deposited polymer membrane which can be dispersed in aqueous carrier liquids to be injected into living beings or administered orally, rectally and urethrally for therapeutic or diagnostic purposes. The properties of the polymeric membrane of the microballoons (elasticity, permeability, biodegradability) can be controlled at will by the selection of polymer, conditions of the interfacial depositions and polymer additives. As possible gases disclosed are physiologically acceptable gases such as CO_2, N_2O, methane, Freon, helium and other rare gases.

Despite the many progresses achieved regarding the stability under storage of aqueous microbubble suspensions, this being either in the precursor or final preparation stage, there still remained until now the problem of vesicle durability when the suspensions are exposed to overpressure, e.g. pressure variations such as that occurring after injection in the blood stream of a patient and consecutive to heart pulses, particularly in the left ventricle. Actually, the present inventors have observed that, for instance in anaesthetised rabbits, the pressure variations are not sufficient to substantially alter the bubble count for a period of time after injection. In contrast, in dogs and human patients, typical microbubbles or microballoons filled with common gases such as air, methane or CO_2 will collapse completely in a matter of seconds after injection due to the blood pressure effect. This observation has been confirmed by others: For instance, S. GOTTLIEB et al. in J. Am. Soc. of Echocardiography 3 (1990) 238 have reported that cross-linked albumin microballoons prepared by the sonication method were losing all echogenic properties after being subjected to an overpressure of 60 Torr. It became hence important to solve the problem and to increase the useful life of suspensions of microbubbles and membrane bounded microballoons under pressure in order to ensure that echographic measurements can be performed in vivo safely and reproducibly.

It should be mentioned at this stage that another category of echogenic image enhancing agents has been proposed which resist overpressures as they consist of plain microspheres with a porous structure, such porosity containing air or a gas. Such microspheres are disclosed for instance in WO-A-91/12823 (DELTA BIOTECHNOLOGY), EP-A-327 490 (SCHERING) and EP-A-458 079 (HOECHST). The drawback with the plain porous microspheres is that the encapsulated gas-filled free space is generally too small for good echogenic response and the spheres lack adequate elasticity. Hence the preference generally remains with the hollow microvesicles and a solution to the collapsing problem was searched.

Disclosure of the Invention

This problem has now been solved by using gases or gas mixtures in conformity with the criteria outlined in the claims. Briefly, it has been found that when the echogenic microvesicles are made in the presence of a gas, respectively are filled at least in part with a gas, having physical properties in conformity with the equation below, then the microvesicles remarkably resist pressure $80 \cdot 10^2 N/m^2$ (>60) Torr after injection for a time sufficient to obtain reproducible echographic measurements:

$$\frac{s_{gas}}{s_{air}} \times \frac{\sqrt{Mw_{air}}}{\sqrt{Mw_{gas}}} \leq 1$$

In the foregoing equation, "s" designates the solubilities in water expressed as the "BUNSEN" coefficients, i.e. as volume of gas dissolved by unit volume of water under standard conditions (1 bar, 25°C), and under partial pressure of the given gas of 1 atm (see the Gas Encyclopaedia, Elsevier 1976). Since, under such conditions and definitions, the solubility of air is .0167, and the square root of its average molecular weight (Mw) is 5.39, the above relation simplifies to:

$$s_{gas}/\sqrt{Mw_{gas}} \leq .0031$$

In the Examples to be found hereafter there is disclosed the testing of echogenic microbubbles and microballoons (see the Tables) filled with a number of different gases and mixtures thereof, and the corresponding resistance thereof to pressure increases, both in vivo and in vitro. In the Tables, the water solubility factors have also been taken from the aforecited Gas Encyclopaedia from "L'Air Liquide", Elsevier Publisher (1976).

The microvesicles in aqueous suspension containing gases according to the invention include most microbubbles and microballoons disclosed until now for use as contrast agents for echography. The preferred microballoons are those disclosed in EP-A-324.938, PCT/EP91/01706 and EP-A-458 745; the preferred microbubbles are those of PCT/EP91/00620; these microbubbles are advantageously formed from an aqueous liquid and a dry powder (microvesicle precursors) containing lamellarized freeze-dried phospholipids and stabilizers; the microbubbles are developed by agitation of this powder in admixture with the aqueous liquid carrier. The microballoons of EP-A-458 745 have a resilient interfacially precipitated polymer membrane of controlled porosity. They are generally obtained from emulsions into microdroplets of polymer solutions in aqueous liquids, the polymer being subsequently caused to precipitate from its solution to form a filmogenic membrane at the droplet/liquid interface, which process leads to the initial formation of liquid-filled microvesicles, the liquid core thereof being eventually substituted by a gas.

In order to carry out the method of the present invention, i.e. to form or fill the microvesicles, whose suspensions in aqueous carriers constitute the desired echogenic additives, with the gases according to the foregoing relation, one can either use, as a first embodiment, a two step route consisting of (1) making the microvesicles from appropriate starting materials by any suitable conventional technique in the presence of any suitable gas, and (2) replacing this gas originally used (first gas) for preparing the microvesicles with a new gas (second gas) according to the invention (gas exchange technique).

Otherwise, according to a second embodiment, one can directly prepare the desired suspensions by suitable usual methods under an atmosphere of the new gas according to the invention.

If one uses the two-step route, the initial gas can be first removed from the vesicles (for instance by evacuation under suction) and thereafter replaced by bringing the second gas into contact with the evacuated product, or alternatively, the vesicles still containing the first gas can be contacted with the second gas under conditions where the second gas will displace the first gas from the vesicles (gas substitution). For instance, the vesicle suspensions, or preferably precursors thereof (precursors here may mean the materials the microvesicle envelopes are made of, or the materials which, upon agitation with an aqueous carrier liquid, will generate or develop the formation of microbubbles in this liquid), can be exposed to reduced pressure to evacuate the gas to be removed and then the ambient pressure is restored with the desired gas for substitution. This step can be repeated once or more times to ensure complete replacement of the original gas by the new one. This embodiment applies particularly well to precursor preparations stored dry, e.g. dry powders which will regenerate or develop the bubbles of the echogenic additive upon admixing with an amount of carrier liquid. Hence, in one preferred case where microbubbles are to be formed from an aqueous phase and dry laminarized phospholipids, e.g. powders of dehydrated lyophilized liposomes plus stabilizers, which powders are to be subsequently dispersed under agitation in a liquid aqueous carrier phase, it is advantageous to store this dry powder under an atmosphere of a gas selected according to the invention. A preparation of such kind will keep indefinitely in this state and can be used at any time for diagnosis, provided it is dispersed into sterile water before injection.

Otherwise, and this is particularly so when the gas exchange is applied to a suspension of microvesicles in a liquid carrier phase, the latter is flushed with the second gas until the replacement (partial or complete) is sufficient for the desired purpose. Flushing can be effected by bubbling from a gas pipe or, in some cases, by simply sweeping the surface of the liquid containing the vesicles under gentle agitation with a stream (continuous or discontinuous) of the new gas. In this case, the replacement gas can be added only once in the flask containing the suspension and allowed to stand as such for a while, or it can be renewed one or more times in order to assure that the degree of renewal (gas exchange) is more or less complete.

Alternatively, in a second embodiment as said before, one will effect the full preparation of the suspension of the echogenic additives starting with the usual precursors thereof (starting materials), as recited in the prior art and operating according to usual means of said prior art, but in the presence of the desired gases or mixture of gases according

to the invention instead of that of the prior art which usually recites gases such as air, nitrogen, CO_2 and the like.

It should be noted that in general the preparation mode involving one first type of gas for preparing the microvesicles and, thereafter, substituting the original gas by a second kind of gas, the latter being intended to confer different echogenic properties to said microvesicles, has the following advantage: As will be best seen from the results in the Examples hereinafter, the nature of the gas used for making the microvesicles, particularly the microballoons with a polymer envelope, has a definitive influence on the overall size (i.e. the average mean diameter) of said microvesicles; for instance, the size of microballoons prepared under air with precisely set conditions can be accurately controlled to fall within a desired range, e.g. the 1 to 10 μm range suitable for echographying the left and right heart ventricles. This not so easy with other gases, particularly the gases in conformity with the requirements of the present invention; hence, when one wishes to obtain microvesicles in a given size range but filled with gases the nature of which would render the direct preparation impossible or very hard, one will much advantageously rely on the two-steps preparation route, i.e. one will first prepare the microvesicles with a gas allowing more accurate diameter and count control, and thereafter replace the first gas by a second gas by gas exchange.

In the description of the Experimental part that follows (Examples), gas-filled microvesicles suspended in water or other aqueous solutions have been subjected to pressures over that of ambient. It was noted that when the overpressure reached a certain value (which is generally typical for a set of microsphere parameters and working conditions like temperature, compression rate, nature of carrier liquid and its content of dissolved gas (the relative importance of this parameter will be detailed hereinafter), nature of gas filler, type of echogenic material, etc.), the microvesicles started to collapse, the bubble count progressively decreasing with further increasing the pressure until a complete disappearance of the sound reflector effect occurred. This phenomenon was better followed optically, (nephelometric measurements) since it is paralleled by a corresponding change in optical density, i.e. the transparency of the medium increases as the bubble progressively collapse. For this, the aqueous suspension of microvesicles (or an appropriate dilution thereof) was placed in a spectrophotometric cell maintained at 25° C (standard conditions) and the absorbance was measured continuously at 600 or 700 nm, while a positive hydrostatic overpressure was applied and gradually increased. The pressure was generated by means of a peristaltic pump (GILSON's Mini-puls) feeding a viable height liquid column connected to the spectrophotometric cell, the latter being sealed leak-proof. The pressure was measured with a mercury manometer calibrated in Torr. The compression rate with time was found to be linearly correlated with the pump's speed (rpm's). The absorbance in the foregoing range was found to be proportional to the microvesicle concentration in the carrier liquid.

Figure 1 is a graph which relates the bubble concentration (bubble count), expressed in terms of optical density in the aforementioned range, and the pressure applied over the bubble suspension. The data for preparing the graph are taken from the experiments reported in Example 4.

Figure 1

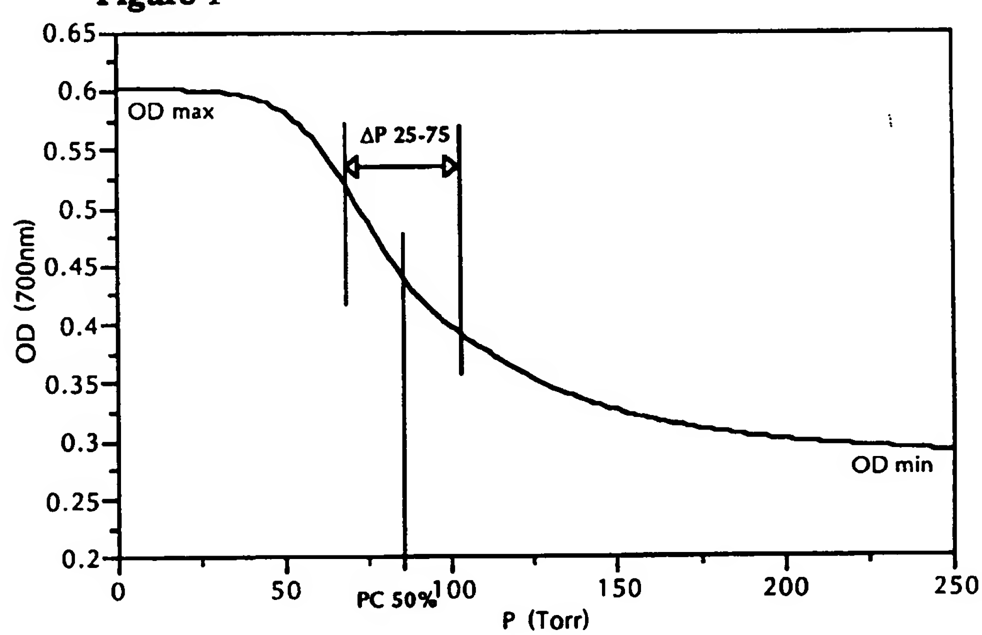


Figure 1 shows graphically that the change of absorbance versus pressure is represented by a sigmoid-shaped

curve. Up to a certain pressure value, the curve is nearly flat which indicates that the bubbles are stable. Then, a relatively fast absorbance drop occurs, which indicates the existence of a relatively narrow critical region within which any pressure increase has a rather dramatic effect on the bubble count. When all the microvesicles have disappeared, the curve levels off again. A critical point on this curve was selected in the middle between the higher and lower optical readings, i.e. intermediate between the "full"-bubble (OD max) and the "no"-bubble (OD min) measurements, this actually corresponding where about 50% of the bubbles initially present have disappeared, i.e. where the optical density reading is about half the initial reading, this being set, in the graph, relative to the height at which the transparency of the pressurized suspension is maximal (base line). This point which is also in the vicinity where the slope of the curve is maximal is defined as the critical pressure PC. It was found that for a given gas, PC does not only depend on the aforementioned parameters but also, and particularly so, on the actual concentration of gas (or gases) already dissolved in the carrier liquid: the higher the gas concentration, the higher the critical pressure. In this connection, one can therefore increase the resistance to collapse under pressure of the microvesicles by making the carrier phase saturated with a soluble gas, the latter being the same, or not, (i.e. a different gas) as the one that fills the vesicles. As an example, air-filled microvesicles could be made very resistant to overpressures (>120 Torr $160 \cdot 10^2 N/m^2$) by using, as a carrier liquid, a saturated solution of CO_2. Unfortunately, this finding is of limited value in the diagnostic field since once the contrast agent is injected to the bloodstream of patients (the gas content of which is of course outside control), it becomes diluted therein to such an extent that the effect of the gas originally dissolved in the injected sample becomes negligible.

Another readily accessible parameter to reproducibly compare the performance of various gases as microsphere fillers is the width of the pressure interval (ΔP) limited by the pressure values under which the bubble counts (as expressed by the optical densities) is equal to the 75% and 25% of the original bubble count. Now, it has been surprisingly found that for gases where the pressure difference $DP = P_{25} - P_{75}$ exceeds a value of about 33-40N/m^2 (25 - 30 Torr), the killing effect of the blood pressure on the gas-filled microvesicles is minimized, i.e. the actual decrease in the bubble count is sufficiently slow not to impair the significance, accuracy and reproducibility of echographic measurements.

It was found, in addition, that the values of PC and ΔP also depend on the rate of rising the pressure in the test experiments illustrated by Fig. 1, i.e. in a certain interval of pressure increase rates (e.g. in the range of several tens to several hundreds of Torr/min), the higher the rate, the larger the values for PC and ΔP. For this reason, the comparisons effected under standard temperature conditions were also carried out at the constant increase rate of 100 Torr/min. It should however be noted that this effect of the pressure increase rate on the measure of the PC and ΔP values levels off for very high rates; for instance the values measured under rates of several hundreds of Torr/min are not significantly different from those measured under conditions ruled by heart beats.

Although the very reasons why certain gases obey the aforementioned properties, while others do not, have not been entirely clarified, it would appear that some relation possibly exists in which, in addition to molecular weight and water solubility, dissolution kinetics, and perhaps other parameters, are involved. However these parameters need not be known to practise the present invention since gas eligibility can be easily determined according to the aforediscussed criteria.

The gaseous species which particularly suit the invention are, for instance, halogenated hydrocarbons like the freons and stable fluorinated chalcogenides like SF_6, SeF_6 and the like.

It has been mentioned above that the degree of gas saturation of the liquid used as carrier for the microvesicles according to the invention has an importance on the vesicle stability under pressure variations. Indeed, when the carrier liquid in which the microvesicles are dispersed for making the echogenic suspensions of the invention is saturated at equilibrium with a gas, preferably the same gas with which the microvesicles are filled, the resistance of the microvesicles to collapse under variations of pressure is markedly increased. Thus, when the product to be used as a contrast agent is sold dry to be mixed just before use with the carrier liquid (see for instance the products disclosed in PCT/EP91/00620 mentioned hereinbefore), it is quite advantageous to use, for the dispersion, a gas saturated aqueous carrier. Alternatively, when marketing ready-to-use microvesicle suspensions as contrast agents for echography, one will advantageously use as the carrier liquid for the preparation a gas saturated aqueous solution; in this case the storage life of the suspension will be considerably increased and the product may be kept substantially unchanged (no substantial bubble count variation) for extended periods, for instance several weeks to several months, and even over a year in special cases. Saturation of the liquid with a gas may be effected most easily by simply bubbling the gas into the liquid for a period of time at room temperature.

Example 1

Albumin microvesicles filled with air or various gases were prepared as described in EP-A- 324 938 using a 10 ml calibrated syringe filled with a 5% human serum albumin (HSA) obtained from the Blood Transfusion Service, Red-Cross Organization, Bern, Switzerland. A sonicator probe (Sonifier Model 250 from Branson Ultrasonic Corp, USA) was lowered into the solution down to the 4 ml mark of the syringe and sonication was effected for 25 sec (energy setting =

8). Then the sonicator probe was raised above the solution level up to the 6 ml mark and sonication was resumed under the pulse mode (cycle = 0.3) for 40 sec. After standing overnight at 4°C, a top layer containing most of the microvesicles had formed by buoyancy and the bottom layer containing unused albumin debris of denatured protein and other insolubles was discarded. After resuspending the microvesicles in fresh albumin solution the mixture was allowed to settle again at room temperature and the upper layer was finally collected. When the foregoing sequences were carried out under the ambient atmosphere, air filled microballoons were obtained. For obtaining microballoons filled with other gases, the albumin solution was first purged with a new gas, then the foregoing operational sequences were effected under a stream of this gas flowing on the surface of the solution; then at the end of the operations, the suspension was placed in a glass bottle which was extensively purged with the desired gas before sealing.

The various suspensions of microballoons filled with different gases were diluted to 1:10 with distilled water saturated at equilibrium with air, then they were placed in an optical cell as described above and the absorbance was recorded while increasing steadily the pressure over the suspension. During the measurements, the suspensions temperature was kept at 25°C.

The results are shown in the Table 1 below and are expressed in terms of the critical pressure PC values registered for a series of gases defined by names or formulae, the characteristic parameters of such gases, i.e. Mw and water solubility being given, as well as the original bubble count and bubble average size (mean diameter in volume).

TABLE 1

Sample	Gas	Mw	Solubility	Bubble count (10^8/ml)	Bubble size (μm)	PC(Torr) ($1,33 \cdot 10^2 N/m^2$)	$S_{gas}/\sqrt{Mw}$
AFre1	CF_4	88	.0038	0.8	5.1	120	.0004
AFre2	$CBrF_3$	149	.0045	0.1	11.1	104	.0004
ASF1	SF_6	146	.005	13.9	6.2	150	.0004
ASF2	SF_6	146	.005	2.0	7.9	140	.0004
AN1	N_2	28	.0144	0.4	7.8	62	.0027
A14	Air	29	.0167	3.1	11.9	53	.0031
A18	Air	29	.0167	3.8	9.2	52	-
A19	Air	29	.0167	1.9	9.5	51	-
AMe1	CH_4	16	.032	0.25	8.2	34	.008
AKr1	Kr	84	.059	0.02	9.2	86	.006
AX1	Xe	131	.108	0.06	17.2	65	.009
AX2	Xe	131	.108	0.03	16.5	89	.009

From the results of Table 1, it is seen that the critical pressure PC increases for gases of lower solubility and higher molecular weight. It can therefore be expected that microvesicles filled with such gases will provide more durable echogenic signals in vivo. It can also be seen that average bubble size generally increases with gas solubility.

Example 2

Aliquots (1 ml) of some of the microballoon suspensions prepared in Example 1 were injected in the jugular vein of experimental rabbits in order to test echogenicity in vivo. Imaging of the left and right heart ventricles was carried out in the grey scale mode using an Acuson 128-XP5 echography apparatus and a 7.5 MHz transducer. The duration of contrast enhancement in the left ventricle was determined by recording the signal for a period of time. The results are gathered in Table 2 below which also shows the PC of the gases used.

TABLE 2

Sample (Gas)	Duration of contrast (sec)	PC (Torr) (x1,33 • $10^2 N/m^2$)
AMe1 (CH_4)	zero	34
A14 (air)	10	53
A18 (air)	11	52
AX1 (Xe)	20	65
AX2 (Xe)	30	89
ASF2(SF_6)	>60	140

From the above results, one can see the existence of a definite correlation between the critical pressure of the gases tried and the persistence in time of the echogenic signal.

Example 3

A suspension of echogenic air-filled galactose microparticles (Echovist® from SCHERING AG) was obtained by shaking for 5 sec 3 g of the solid microparticles in 8.5 ml of a 20% galactose solution. In other preparations, the air above a portion of Echovist® particles was evacuated (0.2 Torr=27N/m^2) and replaced by an SF_6 atmosphere, whereby, after addition of the 20% galactose solution, a suspension of microparticles containing associated sulfur hexafluoride was obtained. Aliquots (1 ml) of the suspensions were administered to experimental rabbits (by injection in the jugular vein) and imaging of the heart was effected as described in the previous example. In this case the echogenic microparticles do not transit through the lung capillaries, hence imaging is restricted to the right ventricle and the overall signal persistence has no particular significance. The results of Table 3 below show the value of signal peak intensity a few seconds after injection.

TABLE 3

Sample No	Gas	Signal peak (arbitrary units)
Gal1	air	114
Gal2	air	108
Gal3	SF_6	131
Gal4	SF_6	140

It can be seen that sulfur hexafluoride, an inert gas with low water solubility, provides echogenic suspensions which generate echogenic signals stronger than comparable suspensions filled with air. These results are particularly interesting in view of the teachings of EP-A-441 468 and 357 163 (SCHERING) which disclose the use for echography purposes of micropartcles, respectively, cavitate and clathrate compounds filled with various gases including SF6; these documents do not however report particular advantages of SF6 over other more common gases with regard to the echogenic response.

Example 4

A series of echogenic suspensions of gas-filled microbubbles were prepared by the general method set forth below:

One gram of a mixture of hydrogenated soya lecithin (from Nattermann Phospholipids GmbH, Germany) and dicetyl-phosphate (DCP), in 9/1 molar ratio, was dissolved in 50 ml of chloroform, and the solution was placed in a 100 ml round flask and evaporated to dryness on a Rotavapor apparatus. Then, 20 ml of distilled water were added and the mixture was slowly agitated at 75°C for an hour. This resulted in the formation of a suspension of multilamellar lipo-

somes (MLV) which was thereafter extruded at 75°C through, successively, 3 μm and 0.8 μm polycarbonate membranes (Nuclepore®). After cooling, 1 ml aliquots of the extruded suspension were diluted with 9 ml of a concentrated lactose solution (83 g/l), and the diluted suspensions were frozen at -45°C. The frozen samples were thereafter freeze-dried under high vacuum to a free-flowing powder in a vessel which was ultimately filled with air or a gas taken from a selection of gases as indicated in Table 4 below. The powdery samples were then resuspended in 10 ml of water as the carrier liquid, this being effected under a stream of the same gas used to fill the said vessels. Suspension was effected by vigorously shaking for 1 min on a vortex mixer.

The various suspensions were diluted 1:20 with distilled water equilibrated beforehand with air at 25°C and the dilutions were then pressure tested at 25°C as disclosed in Example 1 by measuring the optical density in a spectrophotometric cell which was subjected to a progressively increasing hydrostatic pressure until all bubbles had collapsed. The results are collected in Table 4 below which, in addition to the critical pressure PC, gives also the ΔP values (see fig 1).

TABLE 4

Sample No	Gas	M_w	Solubility in H_2O	Bubble count (10^8/ml)	PC (Torr) (x1,33 • $10^2 N/m^2$)	ΔP (Torr) (x1,33 • $10^2 N/m^2$
LFre1	CF_4	88	.0038	1.2	97	35
LFre2	$CBrF_3$	149	.0045	0.9	116	64
LSF1	SF_6	146	.005	1.2	92	58
LFre3	C_4F_8	200	.016	1.5	136	145
L1	air	29	.0167	15.5	68	17
L2	air	29	.0167	11.2	63	17
LAr1	Ar	40	.031	14.5	71	18
LKr1	Kr	84	.059	12.2	86	18
LXe1	Xe	131	.108	10.1	92	23
LFre4	$CHClF_2$	86	.78	-	83	25

The foregoing results clearly indicate that the highest resistance to pressure increases is provided by the most water-insoluble gases. The behavior of the microbubbles is therefore similar to that of the microballoons in this regard. Also, the less water-soluble gases with the higher molecular weights provide the flattest bubble-collapse/pressure curves (i.e. ΔP is the widest) which is also an important factor of echogenic response durability in vivo, as indicated hereinbefore.

Example 5

Some of the microbubble suspensions of Example 4 were injected to the jugular vein of experimental rabbits as indicated in Example 2 and imaging of the left heart ventricle was effected as indicated previously. The duration of the period for which a useful echogenic signal was detected was recorded and the results are shown in Table 5 below in which C_4F_8 indicates octafluorocyclobutane.

TABLE 5

Sample No	Type of gas	Contrast duration (sec)
L1	Air	38
L2	Air	29
LMe1	CH_4	47
LKr1	Krypton	37
LFre1	CF_4	>120
LFre2	$CBrF_3$	92
LSF1	SF_6	>112
LFre3	C_4F_8	>120

These results indicate that, again in the case of microbubbles, the gases according to the criteria of the present invention will provide ultrasonic echo signal for a much longer period than most gases used until now.

Example 6

Suspensions of microbubbles were prepared using different gases exactly as described in Example 4, but replacing the lecithin phospholipid ingredient by a mole equivalent of diarachidoylphosphatidylcholine (C_{20} fatty acid residue) available from Avanti Polar Lipids, Birmingham, Alabama, USA. The phospholipid to DCP molar ratio was still 9/1. Then the suspensions were pressure tested as in Example 4; the results, collected in Table 6A below, are to be compared with those of Table 4.

TABLE 6A

Sample No	Type of gas	Mw of gas	Solubility in water	Bubble count (10^8/ml)	PC (Torr) (x1,33 · $10^2 N/m^2$)	ΔP (Torr) (x1,33 · $10^2 N/m^2$)
LFre1	CF_4	88	.0038	3.4	251	124
LFre2	$CBrF_3$	149	.0045	0.7	121	74
LSF1	SF_6	146	.005	3.1	347	>150
LFre3	C_4F_8	200	.016	1.7	>350	>200
L1	Air	29	.0167	3.8	60	22
LBu1	Butane	58	.027	0.4	64	26
LAr1	Argon	40	.031	3.3	84	47
LMe1	CH_4	16	.032	3.0	51	19
LEt1	C_2H_6	44	.034	1.4	61	26
LKr1	Kr	84	.059	2.7	63	18
LXe1	Xe	131	.108	1.4	60	28
LFre4	$CHClF_2$	86	.78	0.4	58	28

The above results, compared to that of Table 4, show that, at least with low solubility gases, by lengthening the chain of the phospholipid fatty acid residues, one can dramatically increase the stability of the echogenic suspension toward pressure increases. This was further confirmed by repeating the foregoing experiments but replacing the phospholipid component by its higher homolog, i.e. di-behenoylphosphatidylcholine (C_{22} fatty acid residue). In this case, the

resistance to collapse with pressure of the microbubbles suspensions was still further increased.

Some of the microbubbles suspensions of this Example were tested in dogs as described previously for rabbits (imaging of the heart ventricles after injection of 5 ml samples in the anterior cephalic vein). A significant enhancement of the useful in-vivo echogenic response was noted, in comparison with the behavior of the preparations disclosed in Example 4, i.e. the increase in chain length of the fatty-acid residue in the phospholipid component increases the useful life of the echogenic agent in-vivo.

In the next Table below, there is shown the relative stability in the left ventricle of the rabbit of microbubbles (SF_6) prepared from suspensions of a series of phospholipids whose fatty acid residues have different chain lengths (<injected dose: 1 ml/rabbit).

TABLE 6B

Phospholipid	Chain length (C_n)	PC (Torr) (x1,33 • $10^2 N/m^2$)	ΔP (Torr) (x1,33 • $10^2 N/m^2$)	Duration of contrast (sec)
DMPC	14	57	37	31
DPPC	16	100	76	105
DSPC	18	115	95	120
DAPC	20	266	190	>300

It has been mentioned hereinabove that for the measurement of resistance to pressure described in these Examples, a constant rate of pressure rise of 133 • $10^2 N/m^2$ (100 Torr/min) was maintained. This is justified by the results given below which show the variations of the PC values for different gases in function to the rate of pressure increase. In these samples DMPC was the phospholipid used.

Gas sample	PC (Torr) (x1,33 • $10^2 N/m^2$) Rate of pressure increase (Torr/min) (x1,33 • $10^2 N/m^2$,min)		
	40	100	200
SF_6	51	57	82
Air	39	50	62
CH_4	47	61	69
Xe	38	43	51
Freon 22	37	54	67

Example 7

A series of albumin microballoons as suspensions in water were prepared under air in a controlled sphere size fashion using the directions given in Example 1. Then the air in some of the samples was replaced by other gases by the gas-exchange sweep method at ambient pressure. Then, after diluting to 1:10 with distilled water as usual, the samples were subjected to pressure testing as in Example 1. From the results gathered in Table 7 below, it can be seen that the two-steps preparation mode gives, in some cases, echo-generating agents with better resistance to pressure than the one-step preparation mode of Example 1.

TABLE 7

Sample No	Type of gas	Mw of the gas	Solubility in water	Initial bubble count (10^8/ml)	PC (Torr) ($x1{,}33 \cdot 10^2 N/m^2$)
A14	Air	29	.0167	3.1	53
A18	Air	29	.0167	3.8	52
A18/SF_6	SF_6	146	.005	0.8	115
A18/C_2H_6	C_2H_6	30	.042	3.4	72
A19	Air	29	.0167	1.9	51
A19/SF_6	SF_6	146	.005	0.6	140
A19/Xe	Xe	131	.108	1.3	67
A22/CF_4	CF_4	88	.0038	1.0	167
A22/Kr	Kr	84	.059	0.6	85

Example 8

The method of the present invention was applied to an experiment as disclosed in the prior art, for instance Example 1 WO-92/11873. Three grams of Pluronic® F68 (a copolymer of polyoxyethylene-polyoxypropylene with a molecular weight of 8400), 1g of dipalmitoylphosphatidylglycerol (Na salt, AVANTI Polar Lipids) and 3.6 g of glycerol were added to 80 ml of distilled water. After heating at about 80°C, a clear homogenous solution was obtained. The tenside solution was cooled to room temperature and the volume was adjusted to 100 ml. In some experiments (see Table 8) dipalmitoylphosphatidylglycerol was replaced by a mixture of diarachidoylphosphatidylcholine (920 mg) and 80 mg of dipalmitoylphosphatidic acid (Na salt, AVANTI Polar lipids).

The bubble suspensions were obtained by using two syringes connected via a three-way valve. One of the syringes was filled with 5 ml of the tenside solution while the other was filled with 0.5 ml of air or gas. The three-way valve was filled with the tenside solution before it was connected to the gas-containing syringe. By alternatively operating the two pistons, the tenside solutions were transferred back and forth between the two syringes (5 times in each direction), milky suspensions were formed. After dilution (1:10 to 1:50) with distilled water saturated at equilibrium with air, the resistance to pressure of the preparations was determined according to Example 1. The pressure increase rate was $319 \cdot 10^2 N/m^2$ (240 Torr/min.) The following results were obtained:

TABLE 8

Phospholipid	Gas	Pc (mm Hg) ($x1{,}33 \cdot 10^2 N/m^2$)	DP (mm Hg) ($x1{,}33 \cdot 10^2 N/m^2$)
DPPG	air	28	17
DPPG	SF_6	138	134
DAPC/DPPA $_{9/1}$	air	46	30
DAPC/DPPA $_{9/1}$	SF_6	269	253

It follows that by using the method of the invention and replacing air with other gases e.g. SF_6 even with known preparations a considerable improvements i.e. increase in the resistance to pressure may be achieved. This is true both in the case of negatively charged phospholipids (e.g. DPPG) and in the case of mixtures of neutral and negatively charged phospholipids (DAPC/DPPA).

The above experiment further demonstrates that the recognised problem sensitivity of microbubbles and microballoons to collapse when exposed to pressure i.e. when suspensions are injected into living beings, has advantageously been solved by the method of the invention. Suspensions with microbubbles or microballoons with greater resistance against collapse and greater stability can advantageously be produced providing suspensions with better reproducibility

and improved safety of echographic measurements performed in vivo on a human or animal body.

Claims

1. A contrast agent for ultrasonic echography comprising, as a suspension in an aqueous liquid carrier phase, microvesicles filled with a gas or a gas mixture, characterised in that the gas mixture comprises at least one physiologically acceptable halogenated gas whose ratio of solubility in water, expressed in liters of gas by liter of water under standard conditions, to square root of the molecular weight, in daltons, is below 0.0027.

2. The contrast agent of claim 1, wherein the halogenated gas is SF_6, SeF_6 or a freon selected from CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 or C_4F_{10}.

3. The contrast agent of claims 1 or 2 wherein the gas mixture contains a gas selected from air, nitrogen, or CO_2.

4. The contrast agent of claim 3, wherein the microvesicles are microbubbles bounded by an evanescent gas/liquid interfacial closed surface made from dissolved lamellar or laminar phospholipids.

5. The contrast agent of claim 4, wherein at least part of the phospholipids are in the form of liposomes.

6. The contrast agent of claim 5, wherein the liquid carrier phase further contains stabilisers.

7. The contrast agent of claim 4, wherein at least one of the phospholipids is a diacylphosphatidyl compound wherein the acyl group is a C_{16} fatty acid residue or a higher homologue thereof.

8. The contrast agent of claim 3, wherein the microvesicles are microballoons bounded by a material envelope made of an organic polymeric membrane.

9. The contrast agent of claim 8, wherein the polymers of the membrane are selected from polylactic or polyglycolic acid and their copolymers, reticulated serum albumin, reticulated haemoglobin, polystyrene, and esters of polyglutamic and polyaspartic acids.

10. The contrast agent of claim 9, wherein the microvesicles are filled with SF_6.

11. The contrast agent of claim 1, wherein the gas in the microvesicles is selected from SeF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 or C_4F_{10}.

12. A method of making contrast agents comprising the suspensions of gas filled microvesicles in an aqueous liquid carrier phase of claim 1, characterised by forming the microvesicles under an atmosphere of a gas mixture comprising at least one physiologically acceptable halogenated gas whose ratio of solubility in water, expressed in liters of gas by liter of water under standard conditions, to square root of the molecular weight, in daltons, is below 0.0027.

13. A method of making contrast agents comprising the suspensions of gas filled microvesicles in an aqueous liquid carrier phase of claim 1, characterised by filling already made microvesicles with a gas mixture comprising at least one physiologically acceptable halogenated gas whose ratio of solubility in water, expressed in liters of gas by liter of water under standard conditions, to square root of the molecular weight, in daltons, is below 0.0027.

14. The method of claim 12 or 13, wherein the mixture comprises air, nitrogen, or CO_2.

15. The method of claim 12 or 13, wherein the aqueous carrier contains dissolved lamellarized phospholipids and stabilizers stabilizing the microbubbles.

16. The method of claim 13, wherein the microvesicles are formed in two steps, the first step in which the microvesicles or dry precursors thereof are preformed under an atmosphere of a first gas, and then in the second step at least a fraction of the first gas is substituted by the physiologically acceptable halogenated gas.

17. The method of claim 16, wherein the forming of the microvesicles with said gas mixture is effected by alternately subjecting the dry precursors thereof to reduced pressure and restoring the pressure with said gas mixture, and finally dispersing the precursors in a liquid carrier.

18. The method of claim 16, wherein the first gas is air, nitrogen, or CO_2.

19. The method of claims 12 - 18, wherein the halogenated gas is SF_6, SeF_6 or a freon selected from CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 or C_4F_{10}.

20. The method of claim 18, wherein the first gas is completely substituted by the second physiologically acceptable halogenated gas selected from SeF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 and C_4F_{10}.

21. The method of claim 13, wherein the filling of the microvesicles with the gas mixture is effected by flushing the suspension of the microvesicles with the gas mixture.

22. Contrast agent precursors consisting of a dry powder comprising lyophilized liposomes and stabilizers, the powder being dispersible in an aqueous liquid carrier to form echogenic suspensions of gas-filled microvesicles of claim 1, wherein said powder is stored under an atmosphere of a gas mixture comprising at least one physiologically acceptable halogenated gas whose ratio of solubility in water, expressed in liters of gas by liter of water under standard conditions, to square root of the molecular weight, in daltons, is below 0.0027.

23. Contrast agent precursors consisting of a dry powder comprising lyophilized liposomes and stabilizers, the powder being dispersible in an aqueous liquid carrier to form echogenic suspensions of gas-filled microvesicles of claim 1, wherein said powder is stored under an atmosphere of a halogenated gas whose ratio of solubility in water, expressed in liters of gas by liter of water under standard conditions, to square root of the molecular weight, in daltons, is below 0.0027.

24. The contrast agent precursors of claims 22 or 23, wherein the liposomes comprise phospholipids whose fatty acid residues have 16 or more carbon atoms.

25. The contrast agent precursor of claim 22 or 23, wherein the halogenated gas is SF_6, SeF_6 or a freon selected from CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 or C_4F_{10}.

26. The contrast agent precursor of claim 22, wherein the mixture contains air, nitrogen, or CO_2.

27. Contrast agents of claim 1-11 for use in ultrasonic imaging of human or animal body.

28. Use of contrast agent precursors of claims 22-26 for the manufacture of ultrasonic contrast agents.

Patentansprüche

1. Kontrastmittel zur Ultraschallsonographie, enthaltend als Suspension in einer wäßrigen, flüssigen Trägerphase mit Gas oder einer Gasmischung gefüllte Mikrobläschen, dadurch gekennzeichnet, daß die Gasmischung mindestens ein physiologisch verträgliches halogeniertes Gas enthält, dessen Verhältnis von Wasserlöslichkeit, ausgedrückt in Litern Gas pro Liter Wasser unter Standardbedingungen, zur Quadratwurzel des Molekulargewichts, in Dalton, unter 0,0027 liegt.

2. Kontrastmittel gemäß Anspruch 1, worin das halogenierte Gas SF_6, SeF_6 oder ein Freon ausgewählt aus CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 oder C_4F_{10} ist.

3. Kontrastmittel gemäß Anspruch 1 oder 2, worin die Gasmischung ein Gas ausgewählt aus Luft, Stickstoff oder CO_2 enthält.

4. Kontrastmittel gemäß Anspruch 3, worin die Mikrobläschen durch eine vergängliche, geschlossene Gas/Flüssig-Grenzfläche, die aus gelösten lamellaren oder laminaren Phospholipiden hergestellt ist, begrenzte Mikrobläschen sind.

5. Kontrastmittel gemäß Anspruch 4, worin mindestens ein Teil der Phospholipide in Form von Liposomen vorliegt.

6. Kontrastmittel gemäß Anspruch 5, worin die flüssige Trägerphase zusätzlich Stabilisatoren enthält.

7. Kontrastmittel gemäß Anspruch 4, worin mindestens eines der Phospholipide eine Diacylphosphatidylverbindung

ist, worin die Acylgruppe ein C_{16}-Fettsäurerest oder ein höheres Homologes davon ist.

8. Kontrastmittel gemäß Anspruch 3, worin die Mikrobläschen durch eine materielle Hülle, die aus einer organischen Polymermembran hergestellt ist, begrenzte Mikrobläschen sind.

9. Kontrastmittel gemäß Anspruch 8, worin die Polymere der Membran aus Polymilchsäure oder Polyglykonsäure und deren Copolymeren, vernetztem Serumalbumin, vernetztem Hämoglobin, Polystyrol und Estern von Polyglutamin- und Polyasparaginsäure ausgewählt sind.

10. Kontrastmittel gemäß Anspruch 9, worin die Mikrobläschen mit SF_6 gefüllt sind.

11. Kontrastmittel gemäß Anspruch 4 oder 8, worin das Gas in den Mikrobläschen aus SeF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 oder C_4F_{10} ausgewählt ist.

12. Verfahren zur Herstellung von Kontrastmitteln enthaltend die Suspensionen Gas-gefüllter Mikrobläschen in einer wäßrigen flüssigen Trägerphase gemäß Anspruch 1, gekennzeichnet durch das Bilden der Mikrobläschen in einer Atmosphäre einer Gasmischung enthaltend mindestens ein physiologisch verträgliches halogeniertes Gas, dessen Verhältnis von Löslichkeit in Wasser, ausgedrückt in Litern Gas pro Liter Wasser unter Standardbedingungen, zur Quadratwurzel des Molekulargewichts, in Dalton, unter 0,0027 liegt.

13. Verfahren zur Herstellung von Kontrastmitteln enthaltend die Suspensionen Gas-gefüllter Mikrobläschen in einer wäßrigen flüssigen Trägerphase gemäß Anspruch 1, gekennzeichnet durch das Füllen bereits hergestellter Mikrobläschen mit einer Gasmischung enthaltend mindestens ein physiologisch verträgliches halogeniertes Gas, dessen Verhältnis von Löslichkeit in Wasser, ausgedrückt in Litern Gas pro Liter Wasser unter Standardbedingungen, zur Quadratwurzel des Molekulargewichts, in Dalton, unter 0,0027 liegt.

14. Verfahren gemäß Anspruch 12 oder 13, worin die Gasmischung Luft, Stickstoff oder CO_2 enthält.

15. Verfahren gemäß Anspruch 12 oder 13, worin der wäßrige Träger gelöste lamellarisierte Phospholipide und Stabilisatoren enthält, welche die Mikrobläschen stabilisieren.

16. Verfahren gemäß Anspruch 13, worin die Mikrobläschen in zwei Schritten gebildet werden, einem ersten Schritt, in welchem die Mikrobläschen oder trockene Vorstufen davon in einer Atmosphäre eines ersten Gases vorgeformt werden, und einem zweiten Schritt, in dem mindestens ein Teil des ersten Gases durch das physiologisch verträgliche halogenierte Gas ersetzt wird.

17. Verfahren gemäß Anspruch 16, worin das Herstellen der Mikrobläschen mit der Gasmischung dadurch bewirkt wird, daß die trockenen Vorstufen davon abwechselnd reduziertem Druck unterworfen werden und dann der Druck mit der Gasmischung wiederhergestellt wird und schließlich die Vorläufer in einem flüssigen Träger dispergiert werden.

18. Verfahren gemäß Anspruch 16, worin das erste Gas Luft, Stickstoff oder CO_2 ist.

19. Verfahren gemäß den Ansprüchen 12 bis 18, worin das halogenierte Gas SF_6, SeF_6 oder ein Freon ausgewählt aus CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 oder C_4F_{10} ist.

20. Verfahren gemäß Anspruch 18, worin das erste Gas vollständig durch das zweite physiologisch verträgliche halogenierte Gas ersetzt wird, das aus SeF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 oder C_4F_{10} ausgewählt ist.

21. Verfahren gemäß Anspruch 13, worin das Füllen der Mikrobläschen mit der Gasmischung dadurch bewirkt wird, daß die Suspension der Mikrobläschen mit der Gasmischung durchgespült wird.

22. Kontrastmittelvorläufer bestehend aus einem trockenen Pulver, enthaltend lyophilisierte Liposomen und Stabilisatoren, wobei das Pulver in einem wäßrigen flüssigen Träger unter Bildung von ein Echo hervorrufenden Suspensionen Gas-gefüllter Mikrobläschen gemäß Anspruch 1 dispergierbar ist, wobei das Pulver in einer Atmosphäre einer Gasmischung gelagert wird, die mindestens ein physiologisch verträgliches halogeniertes Gas enthält, dessen Verhältnis von Löslichkeit in Wasser, ausgedrückt in Litern Gas pro Liter Wasser unter Standardbedingungen, zur Quadratwurzel des Molekulargewichts, in Dalton, unter 0,0027 liegt.

23. Kontrastmittelvorläufer bestehend aus einem trockenen Pulver, enthaltend lyophilisierte Liposomen und Stabilisatoren, wobei das Pulver in einem wäßrigen flüssigen Träger unter Bildung von ein Echo hervorrufenden Suspensionen Gas-gefüllter Mikrobläschen gemäß Anspruch 1 dispergierbar ist, wobei das Pulver in einer Atmosphäre eines halogenierten Gases gelagert wird, dessen Verhältnis von Löslichkeit in Wasser, ausgedrückt in Litern Gas pro Liter Wasser unter Standardbedingungen, zur Quadratwurzel des Molekulargewichts, in Dalton, unter 0,0027 liegt.

24. Kontrastmittelvorläufer gemäß Anspruch 22 oder 23, worin die Liposomen Phospholipide enthalten, deren Fettsäurereste 16 oder mehr Kohlenstoffatome enthalten.

25. Kontrastmittelvorläufer gemäß Anspruch 22 oder 23, worin das halogenierte Gas SF_6, SeF_6 oder ein Freon ausgewählt aus CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 oder C_4F_{10} ist.

26. Kontrastmittelvorläufer gemäß Anspruch 22, worin die Mischung Luft, Stickstoff oder CO_2 enthält.

27. Kontrastmittel gemäß den Ansprüchen 1 bis 11 zur Verwendung bei Bild gebenden Ultraschallverfahren des menschlichen oder tierischen Körpers.

28. Verwendung eines Kontrastmittelvorläufers gemäß den Ansprüchen 22 bis 26 zur Herstellung eines Ultraschallkontrastmittels.

Revendications

1. Agent de contraste pour l'échographie ultrasonique comprenant, sous forme de suspension dans un liquide porteur aqueux, des microvésicules remplies de gaz ou d'un mélange de gaz, caractérisé en ce que le mélange de gaz comprend au moins un gaz halogéné physiologiquement acceptable pour lequel le quotient de sa solubilité dans l'eau, donnée en litres de gaz par litre d'eau dans les conditions standard, à la racine carrée de son poids moléculaire en daltons est inférieur à 0,0027.

2. Agent de contraste suivant la revendication 1, dans lequel le gaz halogéné est SF_6, SeF_6, ou un fréon choisi parmi CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 et C_4F_{10}.

3. Agent de contraste suivant les revendications 1 ou 2, dans lequel le mélange de gaz contient un gaz choisi parmi l'air, l'azote ou le CO_2.

4. Agent de contraste suivant la revendication 3, dans lequel les microvésicules sont des microbulles bornées par un interface évanescent gaz/liquide fermé constitué par des phospholipides lamellaires ou laminaires dissous.

5. Agent de contraste suivant la revendication 4, dans lequel au moins une partie des phospholipides est en forme de liposomes.

6. Agent de contraste suivant la revendication 5, dans lequel la phase de liquide porteur aqueux contient, en plus, des stabilisants.

7. Agent de contraste suivant la revendication 4, dans lequel au moins un des phospholipides est un composé diacylphosphatidyle dans lequel le groupe acyle est un reste d'acide gras en C_{16} ou d'un homologue supérieur.

8. Agent de contraste suivant la revendication 3, dans lequel les microvésicules sont des microballons limités par une enveloppe de matière faite d'une membrane de polymère organique.

9. Agent de contraste suivant la revendication 8, dans lequel les polymères de la membrane sont choisis parmi les acides polylactique et polyglycolique et leurs copolymères, l'albumine de sérum réticulée, l'hémoglobine réticulée, le polystyrène et des esters d'acides polyglutamique et polyaspartique.

10. Agent de contraste suivant la revendication 9, dans lequel les microvésicules sont remplies de SF_6.

11. Agent de contraste suivant les revendication 4 ou 8, dans lequel le gaz des microvésicules est choisi parmi SeF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 ou C_4F_{10}.

12. Procédé de fabrication d'agents de contraste comprenant les microvésicules de gaz en suspensions dans une phase de liquide porteur aqueux suivant la revendication 1, caractérisé en ce qu'on forme les microvésicules sous une atmosphère gazeuse comprenant au moins un gaz halogéné physiologiquement acceptable pour lequel le quotient de la solubilité dans l'eau donnée en litres de gaz par litre d'eau dans les conditions standard à la racine carrée de son poids moléculaire en dalton est inférieur à 0,0027.

13. Procédé de fabrication d'agents de contraste comprenant les microvésicules de gaz en suspensions dans une phase de liquide porteur aqueux suivant la revendication 1, caractérisé en ce qu'on remplit d'un mélange de gaz des vésicules préfabriquées, ce mélange comprenant au moins un gaz halogéné physiologiquement acceptable pour lequel le quotient de la solubilité dans l'eau donnée en litres de gaz par litre d'eau dans les conditions standard à la racine càrrée de son poids moléculaire en dalton est inférieur à 0,0027.

14. Procédé suivant les revendications 12 ou 13, dans lequel le mélange contient de l'air, de l'azote ou du CO_2.

15. Procédé suivant les revendications 12 ou 13, dans lequel le liquide porteur aqueux contient des phospholipides lamellaires dissous et des stabilisants des microbulles.

16. Procédé suivant la revendication 13, dans lequel on forme les microbulles en deux étapes; dans la première on préforme des microbulles ou des précurseurs secs de celles-ci sous atmosphère d'un premier gaz, et dans la seconde, on substitue au moins un partie de ce premier gaz par le gaz halogéné physiologiquement acceptable.

17. Procédé suivant la revendication 16, dans lequel la formation des microvésicules avec ledit mélange de gaz est effectuée en soumettant en alternance les précurseurs secs à pression réduite, puis rétablissant la pression au moyen dudit mélange de gaz, et finalement en dispersant les précurseurs dans un liquide porteur.

18. Procédé suivant la revendication 16, dans lequel le premier gaz est l'air, l'azote ou le CO_2.

19. Procédé suivant Tes revendications 12-18, dans lequel le gaz halogéné est SF_6, SeF_6, ou un fréon choisi parmi CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 et C_4F_{10}.

20. Procédé suivant la revendication 18, dans lequel le premier gaz est complètement substitué par le second gaz halogéné et physiologiquement acceptable choisi parmi SF_6, SeF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 et C_4F_{10}.

21. Procédé suivant la revendication 13, dans lequel on remplit les microvésicules du mélange de gaz en balayant la suspension des microvésicules par ce mélange.

22. Précurseurs d'agent de contraste consistant en une poudre sèche comprenant des liposomes lyophilisé et des stabilisants, la poudre étant dispersible dans un liquide porteur aqueux de manière à former des suspensions de microvésicules de gaz suivant la revendication 1, caractérisés en ce que ladite poudre est stockée sous atmosphère d'un mélange de gaz comprenant au moins un gaz halogéné physiologiquement acceptable pour lequel le quotient de la solubilité dans l'eau, donnée en litres de gaz par litre d'eau dans les conditions standard, à la racine carrée de son poids moléculaire en daltons est inférieur à 0,0027.

23. Précurseurs d'agent de contraste consistant en une poudre sèche comprenant des liposomes lyophilisé et des stabilisants, la poudre étant dispersible dans un liquide porteur aqueux de manière à former des suspensions de microvésicules de gaz suivant la revendication 1, caractérisés en ce que ladite poudre est stockée sous atmosphère d'un gaz halogéné physiologiquement acceptable pour lequel le quotient de sa solubilité dans l'eau, donnée en litres de gaz par litre d'eau dans les conditions standard, à la racine carrée de son poids moléculaire en daltons est inférieur à 0,0027.

24. Précurseurs d'agent de contraste suivant les revendications 22 ou 23, dans lesquels les liposomes comprennent des phospholipides dont les restes d'acides gras ont 16 atomes de C, ou plus.

25. Précurseurs d'agent de contraste suivant les revendications 22 ou 23, dans lesquels le gaz halogéné est SF_6, SeF_6, ou un fréon choisi parmi CF_4, $CBrF_3$, C_4F_8, $CClF_3$, CCl_2F_2, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, CBr_2F_2 et C_4F_{10}.

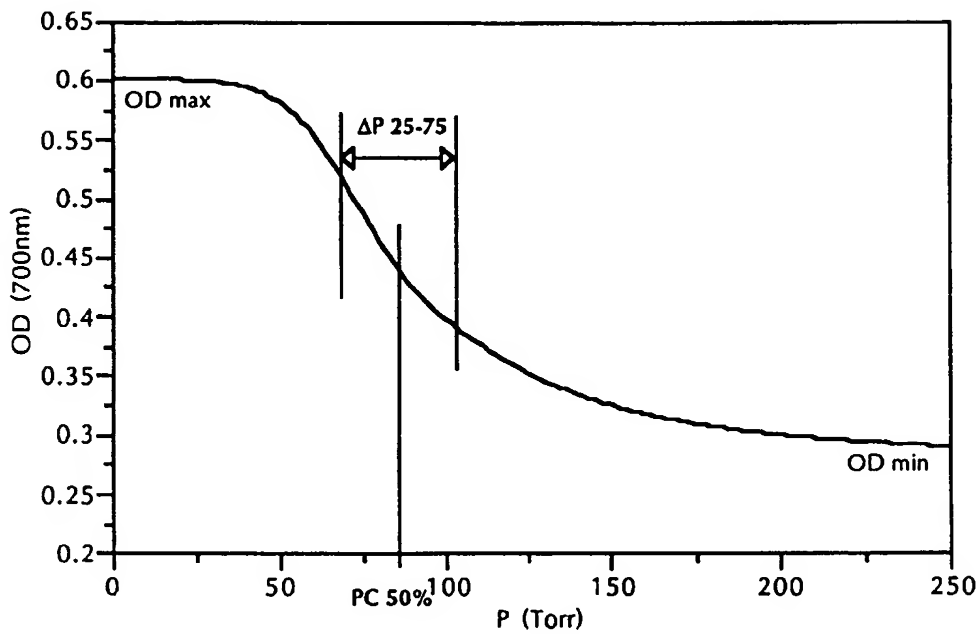
26. Précurseur d'agent de contraste suivant la revendication 22, dans lequel le mélange comprend de l'air, de l'azote

ou du CO_2.

27. Les agents de contraste des revendications 1-11, pour leur utilisation en imagerie par ultrasons du corps humain ou animal.

28. Utilisation des précurseurs d'agent de contraste suivant les revendications 22 -26 pour la fabrication d'agents de contraste pour l'échographie.

Fig 1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0.4
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OD (700nm)
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OD min
0
50
100
150
200
250
PC 50%
P (Torr)